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Effects of an interleukin-1 receptor antagonist on human sleep, sleep-associated memory consolidation, and blood monocytes

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ABSTRACT

Pro-inflammatory cytokines like interleukin-1 beta (IL-1) are major players in the interaction between the immune system and the central nervous system. Various animal studies report a sleep-promoting effect of IL-1 leading to enhanced slow wave sleep (SWS). Moreover, this cytokine was shown to affect hippocampus-dependent memory. However, the role of IL-1 in human sleep and memory is not yet understood. We administered the synthetic IL-1 receptor antagonist anakinra (IL-1ra) in healthy humans (100 mg, subcutaneously, before sleep; $n = 16$) to investigate the role of IL-1 signaling in sleep regulation and sleep-dependent declarative memory consolidation. Inasmuch monocytes have been considered a model for central nervous microglia, we monitored cytokine production in classical and non-classical blood monocytes to gain clues about how central nervous effects of IL-1ra are conveyed. Contrary to our expectation, IL-1ra increased EEG slow wave activity during SWS and non-rapid eye movement (Non-REM) sleep, indicating a deepening of sleep, while sleep-associated memory consolidation remained unchanged. Moreover, IL-1ra slightly increased prolactin and reduced cortisol levels during sleep. Production of IL-1 by classical monocytes was diminished after IL-1ra. The discrepancy to findings in animal studies might reflect species differences and underlines the importance of studying cytokine effects in humans.

1. Introduction

Sleep and the immune system are thought to interact such that sleep promotes immune defense and immune responses to infectious agents convey an enhancing influence on sleep (Besedovsky et al., 2012; Imeri and Opp, 2009). Stimulated by early work that identified a muramyl peptide, i.e., a fragment of bacterial cell walls, as potent sleep promoting factor (Krueger et al., 1982), the idea has been around for a long time that pro-inflammatory cytokines that are released in response to microbial challenge are involved in the homeostatic regulation of sleep, especially of slow wave sleep (SWS) (Krueger and Majde, 1994; Krueger et al., 1995). Interleukin-1 beta (IL-1) and tumor necrosis factor (TNF), two important cytokines mediating inflammatory processes, are considered major players in this context (Krueger et al., 2007). Intracerebroventricular (i.c.v.) injection of IL-1 and TNF enhances non-rapid eye movement (NonREM) sleep duration and slow wave activity in rats, mice and rabbits, whereas inhibition of IL-1 signaling diminishes Non-REM sleep in these species (Krueger et al., 2007; Obál and Krueger, 2003). Whereas initially these cytokines were assumed to originate from peripheral sites, more recent research focused on brain microglia and macrophages as crucial source of IL-1 and TNF, both of which are released during synaptic activity and promote SWS also under normal physiological conditions, i.e., in the absence of any infectious challenge (Krueger et al., 2008). Apart from regulating sleep, microglial pro-inflammatory cytokines are involved in plastic neuronal processes underlying memory formation in the hippocampus (Williamson et al., 2011). Both IL-1 and TNF contribute to synaptic long-term potentiation (LTP), a major mechanism underlying the formation of neuronal memory representations (Ben Menachem-Zidon et al., 2011; del Rey et al., 2013; Gruber-Schoffnegger et al., 2013; Schneider et al., 1998).

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Whereas in animals administration of IL-1 and TNF induced robust enhancements of NonREM sleep with opposite effects after blocking respective receptors (e.g., Opp and Krueger, 1991; Takahashi et al., 1996b, 1997), there is an obvious paucity of studies of such cytokines in humans. Minocycline which directly suppresses microglial activation in an anti-inflammatory manner, decreased SWS and NonREM sleep in humans and animals, respectively (Nonaka et al., 1983; Wisor et al., 2011). In pilot studies, patients with rheumatoid arthritis and obstructive sleep apnea showed a decrease in sleepiness after blocking of IL-1 and TNF-signaling, respectively (Omdal and Gunnarsson, 2005; Vgontzas et al., 2004). In another study, abstinent patients with alcohol dependence showed a robust decrease in REM sleep following treatment with the TNF antagonist etanercept (Irwin et al., 2009). Surprisingly, in these human studies TNF blockade did not alter polysomnographic signs of NonREM sleep (Vgontzas et al., 2004; Irwin et al., 2009), which contrasts with findings in rabbits, where i.c.v. injection of a TNF receptor blocker mainly diminished NonREM sleep (Takahashi et al., 1996b). Although primary effects of pro-inflammatory cytokines on NonREM and SWS have been demonstrated also in humans (e.g., Raison et al., 2010; Späth-Schwalbe et al., 2000), to the best of our knowledge so far no attempts have been made to specifically scrutinize the role of IL-1 for human SWS.

Here, we investigated the effects of s.c. administration of a single dose of the IL-1 receptor antagonist anakinra (IL-1ra) on sleep in healthy humans. As the antagonist crosses the blood–brain barrier (Cawthorne et al., 2011; Clark et al., 2008; Gutierrez et al., 1994), we expected to replicate the suppressive effects on SWS observed after i.c.v. administration of similar IL-1 antagonists in animals (Opp and Krueger, 1991; Takahashi et al., 1997). Considering the well-known consolidating effect of SWS on hippocampus-dependent declarative memory (Diekelmann and Born, 2010) as well as findings that microglial-derived pro-inflammatory cytokines contribute to hippocampal LTP, we also investigated effects of IL-1ra on overnight consolidation of declarative memory (word-pairs, texts). Finally, to gain hints on how central nervous effects of IL-1ra are mediated, we monitored IL-1 production by circulating monocytes, in parallel with sleep and overnight memory consolidation. In this context, we aimed to elucidate which monocyte subpopulation (i.e., either $CD14^{++}CD16^{-}$ or $CD14^{dim}CD16^{++}$ monocytes) can be used as a suitable model of brain microglial IL-1 production, accessible to evaluation in human blood (Anthony et al., 2005; Leone et al., 2006; Varvel et al., 2012).

2. Material and methods

2.1. Subjects

Subjects were 16 healthy men aged on average 22.75 ± 0.93 years (range 18–31 years). Women were not included in this study because of known interactions between sleep and the menstrual cycle (e.g., Baker and Driver, 2007) and, thus, to reduce inter-individual variance, although this limits generalizability of results. Subject numbers were calculated by power analysis based on results from other pharmacological studies using comparable outcome measures (e.g., Feld et al., 2014; Groch et al., 2013). They were non-smokers presenting a normal nocturnal sleep pattern and did not take any medication at the time of the experiments. Acute and chronic illness was excluded by medical history, physical examination, and clinical routine laboratory investigation. The subjects had a regular sleep–wake rhythm for at least 6 weeks before the experiments. Subjects were familiarized with the experimental setting by spending an adaptation night in the laboratory that included the attachment of electrodes for sleep recordings and the insertion of an intravenous (i.v.) forearm catheter for blood sampling. Written informed consent was obtained from each participant, and the study was approved by the local ethics committee.

2.2. Experimental design and procedure

The experiments were conducted in the sleep laboratories of the University Hospital Schleswig–Holstein, Campus Luebeck, Germany, according to a placebo-controlled within-subject crossover design. Each man participated in two experimental conditions. In one condition he was subcutaneously (s.c.) injected with the IL-1ra anakinra (Kineret®, Biovitrum, Sweden; 100 mg dissolved in 0.67 ml saline solution), half-life in plasma 4–7 h (Akash et al., 2013; Chang et al., 2004); in the other condition placebo (saline solution that was visually indistinguishable from anakinra) was injected. Participants, examiners and people assessing the outcomes of the measurements were all blinded to the condition. The order of substance administration was balanced across subjects, who were randomly allocated to one of the two possible orders based on the automated calculation of a random number. Both sessions for a subject were separated by an interval of at least 14 days. The lack of differences in baseline values of IL-1ra between conditions proved that 2 weeks were sufficient for the drug being washed out, although this does not exclude that (undetected) biological effects might have persisted for a much longer time after the substance has been cleared from the body.

On experimental nights, participants arrived at the laboratory at 19:30 h. Following preparations for polysomnographic recordings, they performed (between 21:00 and 22:30 h, always in the same order) on a word pair associates learning task and a text learning task, with a 10-min break between the tasks. After the learning phase and 30 min before lights were turned off (at 23:00 h) to enable sleep, the substance was administered. Blood samples were taken at 20:30, 21:30, 22:30, 23:30 h, and then every hour until 6:30 h for analyses of hormone and IL-1ra concentrations and for flow cytometry. Blood was sampled via an i.v. forearm catheter that was connected to a long thin tube and enabled blood collection from an adjacent room without disturbing the subject's sleep. The total volume of blood sampled during a session was 250 ml.

Subjects were awakened at 7:00 h and then left the lab. During the following day participants engaged in their usual activities. They were instructed to refrain from any stressful mental or physical activities, which were confirmed by a record of their activities during the day. In the evening they returned to the lab at 20:00 h and retrieval of the memory tasks was tested – in reverse order of learning and a last blood sample was taken. Possible side effects of IL-1ra administration were excluded by a standardized interview and questionnaire assessing drug awareness and symptoms like dizziness, pain, headache, etc. on the morning after sleep and after subjects returned to the lab in the evening. There were no significant side effects.

2.3. Polysomnography, sleep EEG analyses, and subjective sleep quality

Standard polysomnographic recordings were obtained including electroencephalographic (EEG) recordings from electrodes attached at C3 and C4 (according to the international 10–20 system, referenced to electrodes attached to the mastoids) as well as electrooculographic and electromyographic recordings. Signals were amplified (Brain Amp, Brain Products, Germany) and digitized, with the EEG sampled at a rate of 200 Hz and filtered between 0.16 and 70 Hz. Sleep stages were determined off-line for subsequent 30-s recording epochs following standard criteria (Rechtschaffen and Kales, 1968). Total sleep time (TST), and the time spent in the different sleep stages (wake; stages 1, 2, 3, and

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4; and REM sleep) were calculated in minutes and as percentage of TST. Sleep onset was defined by the first occurrence of stage 1 sleep followed by stage 2 sleep. Sleep onset latency was determined with reference to lights off. SWS was defined as the sum of time spent in stages 3 and 4 sleep. Latencies of SWS and REM sleep were assessed with respect to sleep onset.

For a more fine-grained analysis of NonREM sleep, EEG power spectra were calculated applying Fast Fourier Transformation (FFT; Vision Analyzer, Brain Products, Germany) on succeeding 10.24-s (2048 data points) epochs of NonREM sleep (S2, S3, S4) or SWS (S3, S4). Only epochs free of movement or muscle artifacts were used. Mean power density was determined for the 0.6–4 Hz (slow wave activity) and the 12–15 Hz (spindle) frequency bands. Mean power density was determined for the whole night, separately for the first and second night half.

Subjective sleep quality was evaluated using a modified version of the sleep questionnaire SF-A/R (Görtelmeyer, 2011). Mood was measured using the Positive and Negative Affect Schedule (PANAS). Participants responded to items (e.g., "Do you momentarily feel scared?") on a 5-point Likert scale ranging from 1 = "not at all" to 5 = "very much". Subjective sleepiness was assessed with the Stanford Sleepiness Scale (SSS).

2.4. Memory tests

A declarative verbal paired associates task was applied that required learning a list of 40 pairs of semantically related words (e.g., clock–church). Different word lists were used on the subject's two experimental nights. During the learning phase, the word pairs were presented sequentially on a computer screen, each for 3 s, separated by inter-stimulus intervals of 500 ms. After presentation of the entire list, performance was tested using a cued recall procedure, i.e., the first word (cue) of each pair was presented and the subject had to name the associated second word (response). The correct response word was then displayed for 1 s, regardless of whether the response was correct or not, to allow re-encoding of the correct word pair. The cued recall procedure was repeated until the subject reached a criterion of 60% correct responses. The number of word pairs recalled at retrieval testing, relative to the number of word pairs recalled at the criterion trial during the learning phase (with learning performance set to 100%), served as a measure of overnight retention.

In addition, a text learning task was applied. Two standardized neutral German texts ("Bronze" and "Fashion") which were validated in previous studies (Schürer-Necker, 1994) were used on the subject's two experimental nights. In the learning phase, the subjects were instructed to carefully read the text, which was written on a sheet of paper, within 4 min and to memorize its content as detailed as possible. After reading, subjects rated the text on 7-point Likert scales on the dimensions: comprehensible–incomprehensible, interesting–uninteresting, difficult–easy, neutral–emotional, harmless–startling, important–unimportant, vivid–abstract, amusing–serious, boring–arousing, familiar–unfamiliar, positive–negative. Thereafter, in an immediate free recall test, the participants were asked to write down the previously read text as accurately as possible, without time restriction. This immediate test served to determine how much information was initially encoded, thereby providing an individual baseline value for recall performance assessed at later retrieval testing after sleep on the next morning. The number of correct content words recalled at retrieval testing, relative to the number of correct content word recalled during the learning phase (with learning performance set to 100%), served as a measure of overnight retention. At retrieval testing, in addition to free recall performance, memory for the temporal order in the texts was assessed. In this test, 12 pairs of words were presented with one word of the pair representing a content word of the text and the other one a synonym. Subjects were required to select the correct content words and order these words according to the sequential order in which they occurred in the text. Recall of sequential order was determined by a deviation score, that is, the distance of the remembered sequence position for a content word from its actual position in the story (Wilhelm et al., 2011).

2.5. Analyses of hormones and IL-1ra

Blood samples were kept frozen until assay. ACTH, cortisol, growth hormone (GH), and prolactin were measured using the Immulite assay (Siemens Healthcare Diagnostics, Erlangen, Germany). Epinephrine and norepinephrine were measured by HPLC (Chromsystems GmbH, Graefelfing, Germany). IL-1ra was detected in plasma with an assay from Quantikine (R&D Systems, Wiesbaden, Germany). Assay sensitivities were as follows: ACTH: 9 pg/ml, cortisol: 0.2 μg/dl, epinephrine: 15 ng/l, GH: 0.1 ng/ml, IL-1ra: 18.3 pg/ml, norepinephrine: 15 ng/l, and prolactin: 0.5 ng/ml. Intra-assay and inter-assay coefficients of variations were all <12.7%.

2.6. Flow cytometric analyses

We measured cytokine production in two monocyte subpopulations, i.e., classical monocytes ($CD14^{++}CD16^{-}$) and non-classical monocytes ($CD14^{dim}CD16^{++}$), without further stimulation as previously described (Selkirk et al., 2009). Blood was transferred into a sodium heparin tube which was pre-spiked with Brefeldin A and which was then immunostained with anti-CD14-PerCP and anti-CD16-V500. FACS Permeabilizing Solution (BD Biosciences, Heidelberg, Germany) was added and nonspecific binding blocked using Human TruStain (FcX Biozol Diagnostica, München, Germany). After intracellular staining with anti-IL-1ra-PE, anti-IL-1β-FITC (BD Biosciences, Heidelberg, Germany) and anti TNF-APC (BioLegend, San Diego, California), blood was incubated for 30 min. 2 ml special washing buffer (Cell Wash) were then added and washed out with centrifugation at 500g for 10 min. Finally, the pellet was re-suspended in 300 μl PBS, which included 2% of paraformaldehyde. At least 10,000 $CD14^{+}$ cells were acquired and subsequently analyzed for the production of intracellular IL-1, IL-1ra and TNF on an LSR II flow cytometer (BD Biosciences, Heidelberg, Germany). Cytokine production was quantified as the percentage of cytokine-positive cells as well as by analyzing the respective median fluorescence intensity (MFI).

2.7. Statistical analyses

Statistical analysis was based on analysis of variance (ANOVA), including repeated-measures factors for the administered substance (IL-1ra versus placebo, "Substance") and for time points of measurements ("Time"). EEG data, hormones, and immune parameters were analyzed for the total nights and, in addition, separately for the first and second half of the night. Analyses of hormones and immune parameters included baseline measures (i.e., the average of the first three samples before drug administration) as covariate when appropriate. There were no drop outs during the study, i.e., all 16 subjects completed both conditions. However, sample sizes were reduced to $n = 10$–15 for the analyses of sleep stages, EEG power, hormone concentrations and immune parameters, due to the loss of EEG files (in 2 subjects), EEG artifacts (3 subjects), or problems with blood sampling (3 subjects; please, refer to figure legends for specific n). A CONSORT flow chart summarizes the flow of the subjects at each phase (Supplementary Fig. 1). Degrees of freedom were corrected using the Greenhouse–Geisser procedure. Planned post hoc t-tests were applied to analyze differences at single time points once ANOVA indicated significant effects. Values

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$p \leqslant 0.05$ were considered significant. Data are presented as means ± SEM.

3. Results

3.1. Polysomnography, sleep EEG analyses, and subjective sleep quality

Compared with placebo, IL-1ra did not change TST and the absolute or percent time spent in the different sleep stages. Also, latency of SWS was comparable in both substance conditions (all $p > 0.28$; Table 1). A more fine-grained evaluation of EEG power during periods of SWS and NonREM sleep indicated that IL-1ra enhanced slow wave activity (0.6–4 Hz). For the analysis of SWS the effect was highly significant during the first night half ($F_{(1,10)} = 24.12$, $p = 0.001$ for main effect of Substance, Fig. 1), for the analyses of NonREM sleep (including periods of stage 2 sleep) the effect was most pronounced in the second night-half ($F_{(1,11)} = 6.02$, $p = 0.032$ for main effect of Substance). Fast spindle (12–15 Hz) activity did not differ between conditions ($p > 0.22$). There were also no effects of IL-1ra on subjective sleep quality, sleepiness or mood assessed in the next morning (Table 1).

3.2. Memory tests

Administration of IL-1ra did not affect the retention of declarative memories for word pairs or texts. There were no differences between conditions in the percentage of recalled word pairs (Placebo: 101.57 ± 2.53%; IL-1ra: 102.85 ± 2.59%, $p = 0.70$), the percentage of recalled content words of the text (Placebo: 89.75 ± 2.41%, IL-1ra: 85.24 ± 3.72%, $p = 0.35$), or in the deviation score assessing memory for the temporal order in the texts (Placebo: 27.94 ± 2.77, IL-1ra: 28.56 ± 2.33, $p = 0.86$) during the retrieval phase. Additional analyses including IL-1ra concentration at retrieval testing as covariate likewise did not reveal any differences in recall performance between the substance conditions (all $p > 0.415$), thus excluding a confounding influence from residual IL-1ra activity present during retrieval. Immediate recall of the word pair associates (Placebo: 30.31 ± 1.08, IL-1ra: 29.13 ± 1.09; number of trials to reach the criterion: Placebo: 2.13 ± 0.29, IL-1ra: 1.88 ± 0.24) as well as of the texts (Placebo: 31.38 ± 2.39, IL-1ra: 31.5 ± 4.17) at the end of the learning phase before retention sleep were also comparable for both substance conditions ($p \geqslant 0.32$, for all relevant comparisons).

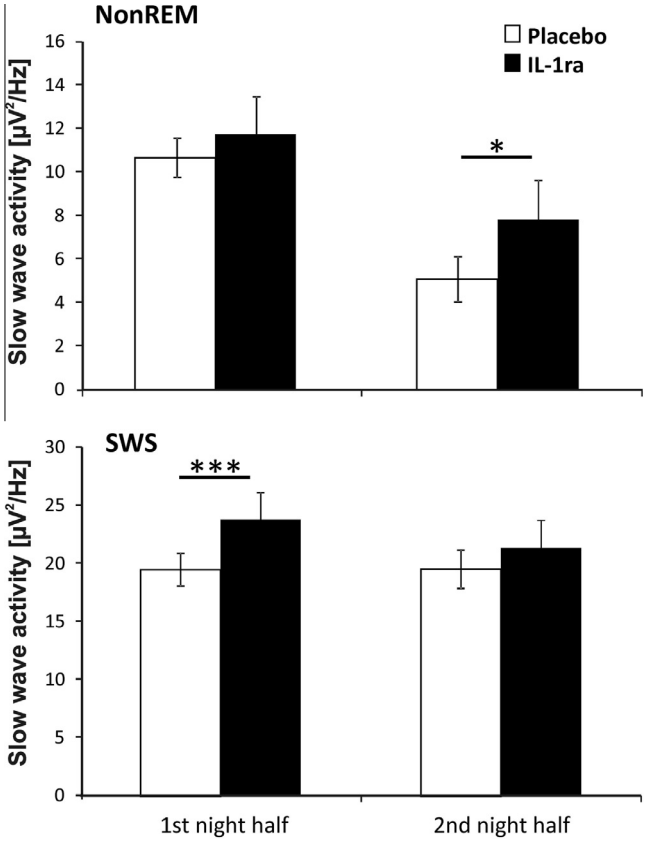

Fig. 1. Slow wave activity during Non-rapid eye movement (NonREM) sleep and slow wave sleep (SWS) after administration of IL-1ra versus placebo. Slow wave activity (power density in μV^2/Hz) during NonREM sleep including sleep stages 2, 3 and 4 (upper panel) and during periods of SWS (lower panel) following administration of IL-1ra (black bars) and placebo (white bars) for the first and second half of the night. Means ± SEM are indicated, $n = 11–14$, $^{*}p < 0.05$, $^{***}p < 0.001$ for pairwise comparisons between IL-1ra and placebo conditions.

Table 1
Sleep and mood.

	IL-1ra		Placebo	
	Means	SEM	Means	SEM
TST, min	424.47	4.64	414.13	12.40
W, %	3.79	1.42	1.63	0.41
S1, %	7.75	1.29	7.17	1.06
S2, %	60.79	2.49	62.04	1.59
S3, %	9.40	0.70	9.73	0.68
S4, %	1.47	0.68	1.43	0.73
REM, %	16.41	1.09	17.70	1.47
SWS, %	10.87	1.14	11.17	1.11
Sleep onset latency, min	25.10	2.82	26.40	6.07
SWS latency, min	27.60	10.04	19.77	0.90
REM latency, min	93.37	9.85	70.40	2.44
SQ	3.02	0.16	3.15	0.19
FRS	2.63	0.16	2.59	0.18
Positive affect	18.50	1.72	18.64	1.52
Negative affect	13.29	1.00	13.43	0.80
Subjective sleepiness	5.14	0.25	4.64	0.31

Percentage of total sleep time (TST) spent awake (W) and in sleep stages 1–4 (S1–S4), slow wave sleep (SWS), and rapid eye movement (REM) sleep, latency of sleep onset (with reference to lights off), and of SWS and REM sleep (with reference to sleep onset), sleep quality (SQ), feeling of refreshment after sleep (FRS), positive and negative affect (on the PANAS) and subjective sleepiness (SSS) measured in the morning after sleep. Means (±SEM) are given for the IL-1ra and placebo conditions. There were no significant differences between conditions.

3.3. Blood hormone and IL-1ra concentrations

IL-1ra administration, as expected, strongly increased IL-1ra blood concentrations throughout the night, and also on the next day ($F_{(1,8)} = 237.54$, $p < 0.001$ for main effect of Substance, Fig. 2) with this prolonged increase diverging from previous pharmacokinetic studies (Chang et al., 2004). Compared with placebo, IL-1ra increased prolactin levels during the first half of the night ($F_{(1,12)} = 6.35$, $p = 0.027$ for main effect of Substance) and decreased cortisol levels ($F_{(1,13)} = 4.96$, $p = 0.044$ for main effect of Substance). IL-1ra also slightly decreased ACTH levels, although this effect was not significant. There were no systematic effects of the antagonist on growth hormone and catecholamine concentrations (data not shown).

3.4. Monocytes and expression of IL-1, TNF and IL-1receptor antagonist (IL-1ra)

Under normal conditions, i.e., following placebo administration, percentages of $CD14^{++}CD16^{-}$ monocytes producing IL-1ra or TNF remained stable across the night, whereas percentages of IL-1 producing $CD14^{++}CD16^{-}$ monocytes declined (from 6.85% at 20:30 h to 4.88% at 6:30 h, $F_{(10,120)} = 4.82$, $p = 0.002$ for main effect of Time).

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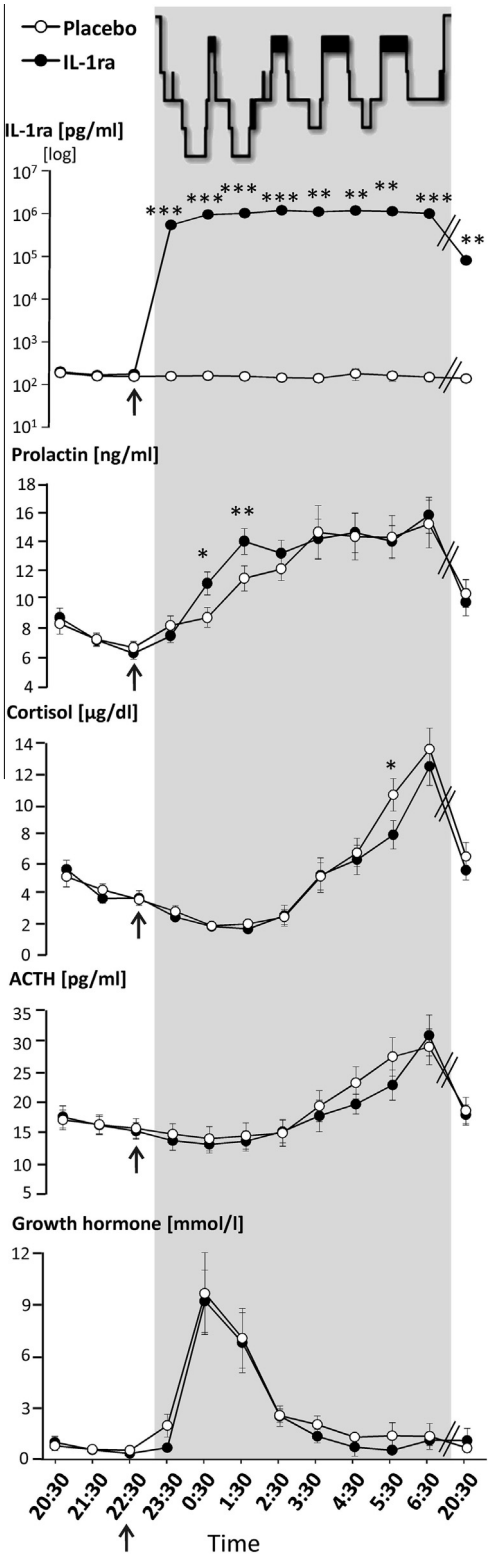

Fig. 2. IL-1ra and hormone serum levels after administration of IL-1ra. Blood serum concentrations of IL-1ra, prolactin, cortisol, ACTH, and growth hormone following administration of IL-1ra (thick lines, black circles) and placebo (thin lines, open circles). Means ± SEM are indicated, $n = 10–15$, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ for pairwise comparisons between IL-1ra and placebo conditions. Values are adjusted to the first three time points (baseline) based on covariance analysis. Gray area indicates time in bed, top panel illustrates prototypical sleep profile, arrows indicate time of substance injection.

Percentages of $CD14^{dim}CD16^{++}$ monocytes producing IL-1, IL-1ra or TNF all rose across nighttime ($F_{(10,120)} > 3.15$, $p < 0.024$ for main effect of Time). The distinct time courses in cytokine production confirm that the two kinds of monocytes represent distinct subtypes that are differentially regulated during the sleep–wake cycle (Dimitrov et al., 2007).

Administration of IL-1ra increased the percentage of $IL\text{-}1ra^{+}$ $CD14^{++}CD16^{-}$ monocytes ($F_{(1,12)} = 15.39$, $p = 0.002$ for main effect of Substance) and the MFI (per cell) of IL-1ra ($F_{(1,11)} = 19.56$, $p = 0.001$ for main effect of Substance) in these cells, which might simply reflect the binding of IL-1ra to IL-1 receptors on these cells. Interestingly, IL-1ra concurrently decreased MFI for IL-1 in these cells during the first night-half ($F_{(1,11)} = 11.42$, $p = 0.006$ for main effect of Substance; Fig. 3). In $CD14^{dim}CD16^{++}$ monocytes, IL-1ra did not affect production of IL-1ra, IL-1, or TNF ($p > 0.125$).

4. Discussion

Studies in rodents and rabbits using blockers and activators of IL-1 signaling have provided consistent evidence that IL-1 promotes sleep, and specifically SWS (Krueger et al., 2007; Obál and Krueger, 2003). Animal studies also revealed that this cytokine supports memory formation and underlying synaptic plasticity (del Rey et al., 2013; Goshen et al., 2007; Schneider et al., 1998; Spulber et al., 2009). In the present study, we tested the effects of inhibiting IL-1 signaling by the IL-1ra anakinra on sleep and sleep-dependent memory consolidation for the first time in healthy humans. We expected that, like in animals, in the human subjects of our study, IL-1ra would diminish SWS and disturb sleep-dependent memory consolidation. Unexpectedly, the main finding of this study was that IL-1ra did not diminish SWS, but even enhanced EEG slow wave activity. Slow wave activity (0.6–4 Hz) is the hallmark of SWS and is a measure of SWS intensity. The effect was most pronounced in the early part of sleep, but was observed for NonREM sleep including lighter sleep stage 2 also in the second half of the night. We did not find any change in retention of declarative memory after IL-1ra administration. However, we found significant changes in sleep-associated hormone secretion: prolactin release was enhanced during the early night and cortisol release was reduced during the late night after IL-1ra. In classical monocytes, IL-1ra reduced IL-1 production, with this effect emerging with some hours delay.

Overall the pattern of changes indicates that administration of the IL-1ra was effective. Yet, why does the outcome of the present experiments in humans diverge so strongly from the findings of previous animal studies? It could be argued that the IL-1ra after s.c. injection did not cross the blood–brain barrier and so did not reach the brain. However, there is consistent evidence, although from animal studies, that anakinra crosses the blood–brain barrier after peripheral administration presumably through a saturable transport process (Gutierrez et al., 1994; Skinner et al., 2009). Confirmatory evidence that anakinra reaches the brain in humans has been so far obtained only in patients with subarachnoid hemorrhage (Clark et al., 2008; Galea et al., 2011). In line with previous findings blood levels of IL-1ra at baseline were ~200 pg/ml and then showed a 10,000 fold increase after anakinra administration (Chang et al., 2004; Frey et al., 2007). Although with peripheral administration levels of IL-1ra achieved per gram brain tissue are only 0.01–0.6% of the injected dose, such levels are within the range of drugs known to produce strong neuromodulatory actions (e.g., Cawthorne et al., 2011; Clark et al., 2008; Gutierrez et al., 1994). Accordingly, peripheral administration of IL-1ra proved to exert potent anti-inflammatory actions in the brain in animals and humans (Clark et al., 2008; Garcia et al., 1995; Relton et al., 1996). Altogether, this excludes that the systemic route of administration per se can explain the divergence in the findings here and in previous animal studies. On the contrary, it seems justified to

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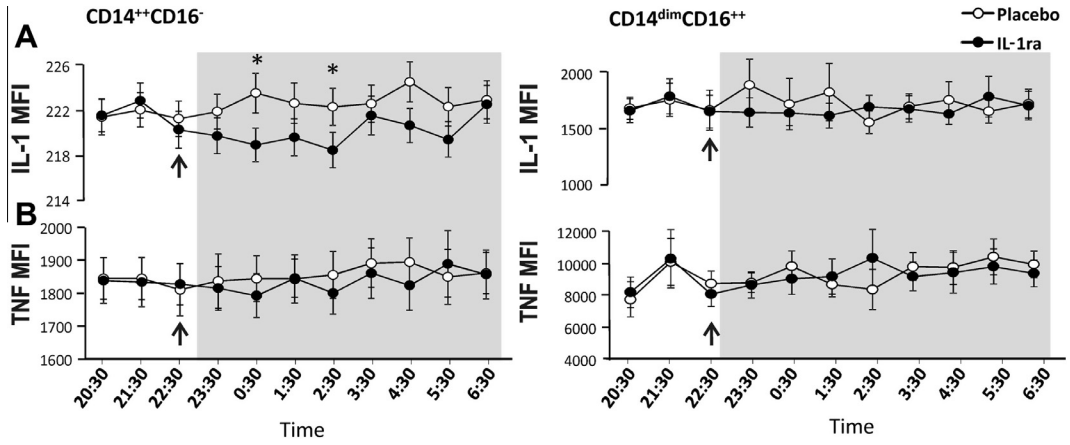

Fig. 3. IL-1 and TNF production in classical and non-classical monocytes. Median fluorescence intensity (MFI) for (A) IL-1 and (B) TNF on classical ($CD14^{++}CD16^{-}$) and non-classical ($CD14^{dim}CD16^{++}$) monocytes following administration of IL-1ra (thick lines, solid circles) and placebo (thin lines, open circles). Means ± SEM are indicated, $n = 13$, $^{*}p < 0.05$ for pairwise comparisons between IL-1ra and placebo conditions. Values are adjusted to the first three time points (baseline) based on covariance analysis. Gray area indicates time in bed, arrows indicate time of substance injection.

assume that our dose of 100 mg IL-1ra s.c., which was based on clinical standard criteria for the treatment of rheumatoid arthritis (Chang et al., 2004) and led to very high plasma levels within 2 h after administration, effectively blocked the action of endogenous IL-1 in the brain. Still this assumption needs to be scrutinized by direct measures of central nervous IL-1ra levels, e.g., in cerebrospinal fluid, which were not performed here.

The dose of IL-1ra administered resulted in clearly supra-physiological concentrations of the antagonist in the blood, and IL-1ra increased the release of prolactin and reduced cortisol levels. Thus, rather than directly acting on the central nervous system, IL-1ra might have increased slow wave activity via inducing specific systemic hormonal changes. Indeed, the increase in prolactin after IL-1ra is consistent with previous studies showing that IL-1 inhibits prolactin secretion (Bernton et al., 1987) and that IL-1ra can increase prolactin secretion (Tanaka et al., 2000). The latter study used *in vitro* stimulation of pituitary cells, indicating that prolactin release can basically result from blood-borne IL-1ra. Prolactin secretion is known to run in parallel with slow wave activity during SWS in healthy men (Spiegel et al., 1995) and high serum levels of prolactin in breast feeding mothers were associated with increases in slow wave activity (Nishihara et al., 2004). However, these findings are correlative in nature. Importantly, animal studies administering prolactin and studies in prolactin knockout mice revealed that the hormone enhances REM sleep, but does not induce any consistent change in SWS (Obál et al., 2005; Zhang et al., 1999). Against this backdrop it is unlikely that enhanced prolactin mediated the effects of IL-1ra in the present study. Rather, the joint increase in EEG slow wave activity and prolactin concentrations after IL-1ra speaks for the view that the antagonist acts on a central nervous mechanism naturally coupling both phenomena.

Decreased cortisol release after IL-1ra can be likewise excluded as factor mediating the enhancing effects of IL-1ra on slow wave activity. This decrease is well in line with findings in animals of a stimulatory effect of IL-1 on hypothalamus–pituitary–adrenal secretory activity (Besedovsky et al., 1986; Gadek-Michalska and Bugajski, 2010; Uehara et al., 1987). However, here the decrease in cortisol concentration was rather small, without concomitant robust decreases in ACTH concentrations, and limited to the second night-half, whereas increases in slow wave activity were most pronounced during the first night-half. Also, increases in early night SWS were found to be a consequence of increases rather than decreases in blood cortisol levels (Born et al., 1989). Because SWS blocks pituitary-adrenal secretory activity (Bierwolf et al., 1997) the decrease in cortisol following IL-1ra might rather be viewed as a consequence of the central nervous enhancement in slow wave activity after IL-1ra administration.

To the best of our knowledge, so far the specific effects of IL-1ra on spontaneous sleep has been examined by only one study in rabbits which revealed a transient reduction in the duration of Non-REM sleep in the first hour after i.c.v. injection of an intermediate dose (~0.025 mg/kg), but not after a high dose (~0.25 mg/kg) of the antagonist (Opp and Krueger, 1991). Animal studies using other methods of blocking IL-1 signaling (like antibodies against IL-1) showed even more robust reductions of Non-REM sleep both after central and peripheral administration of the employed substance (Opp and Krueger, 1994a,b; Takahashi et al., 1996a, 1997). Moreover, some animal studies indicated increases in slow wave activity following enhancing IL-1 signaling and decreased slow wave activity following disruption of IL-1 signaling (Opp and Krueger, 1994a; Takahashi et al., 1996a), although occasionally discrepant results were obtained especially following intraperitoneal administration of IL-1 in rodents (Fang et al., 1998; Hansen and Krueger, 1997). Overall, these results in rats, mice and rabbits appear to be in stark contrast to our finding of increased slow wave activity after IL-1ra administration with no significant effects on SWS duration. This might reflect species specificity of the effect of IL-1ra, although species specificities in the organization of SWS might also be of relevance. For example, in humans SWS is embedded in lighter NonREM sleep stages which are not differentiated in rodents. Species specificities between humans and animals, and also between mice, rats and rabbits have been observed for numerous other immune factors (Mestas and Hughes, 2004). Indeed, several previous studies also found distinct species differences in the impact of cytokine administration and infections on sleep architecture (Irwin et al., 2009; Pollmächer et al., 2000; Raison et al., 2010; Zamarrón et al., 2004).

Brain microglia has been identified as a crucial source of IL-1 and TNF promoting SWS (Krueger et al., 2008; Rothwell, 1999). Because it is not possible to study human microglia *in vivo,* we measured cytokine expression in monocyte subsets, based on evidence suggesting that monocytes, which share the same precursor cells as microglia, might be a potential model for microglial IL-1 production (Anthony et al., 2005; Leone et al., 2006; Varvel et al., 2012). Indeed, in the placebo condition IL-1 producing classical monocytes showed the expected time course with peak percent-

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ages during early sleep and low percentages after sleep, thus mimicking the reported temporal dynamics of IL-1 in brain parenchyma and cerebrospinal fluid (Cearley et al., 2003; Lue et al., 1988; Taishi et al., 1997). IL-1ra selectively decreased production of IL-1 in these classical $CD14^{++}CD16^{-}$ monocytes, but not in non-classical $CD14^{dim}CD16^{++}$ monocytes. The pattern is in line with the well-known stimulatory effect of IL-1 on its own production (Dinarello et al., 1987), suggesting the presence of such positive feedback loop for the production of IL-1 also in classical monocytes. It fits also with observations by Conti et al. (1992) of an inhibition of IL-1 in human monocytes after *in vitro* administration of IL-1ra as well as with observations by Burger et al. (2009) of reduced IL-1 production in multiple sclerosis patients exhibiting simultaneously increased IL-1ra levels. Collectively, these findings imply a twofold synergistic action of IL-1ra in the brain, namely by directly blocking neuronal IL-1 receptors and by additionally decreasing brain IL-1 production in microglia.

Overall, our findings indicate that IL-1ra strengthens SWS by enhancing EEG slow wave activity, with a concomitant increase in prolactin release. The effects very likely reflect central nervous actions of IL-1ra involving both direct actions on neuronal IL-1 receptors as well as a decrease in brain borne IL-1. In diverging from animal studies these findings suggest species specificities to play a role in the effects of IL-1 on sleep. However, this conclusion requires further experimentation, particularly in light of the fact that our study is based on testing of just one specific drug administered through one specific route at only one dose, inducing supra-physiological IL-1ra concentrations in blood that might have indirectly impacted brain function. For a clearer differentiation between the effects of IL-1 on sleep in the different species, different doses and routes of administration need to be tested also in humans. For example the intranasal route of administration might allow for testing direct central nervous effects of the IL-1ra in healthy volunteers (Born et al., 2002). Beyond this issue, the presence of species specificities in the effects of IL-1 eventually underlines the urgent need of directly probing cytokine functions in the human being.

Conflict of interest statement

All authors declare that there are no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbi.2014.11.012.

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